

Review

What do the X and Y chromosomes tell us about sex and gender in forensic case analysis?

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Abstract

Sex determination can be particularly crucial in forensic casework such as rape cases or cases of missing persons. Biological traces have to be genetically typed and the classification of the sex is of great importance for further investigations. Lately, several papers were published on reliability of sex determination by genetic typing of amelogenin gene-specific fragments. Problems may arise not only from false detection (or non-detection) of amelogenin-specific fragments, but also in cases of chimerism (bone marrow transplants) or micro chimerism (pregnant women carrying male fetuses), and from the possible discrepancies between the biological gender and the (forensic relevant) legal gender in the personal identity documents. The phenotype based classification of the legal gender may contradict the genetic sex under several conditions as there are genetic diversity, intersex conditions and transsexualism. The forensic relevance of the possible misinterpretation (sex is not necessarily legal gender) should not be underestimated.

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1. Introduction

Genetic scanning of available traces and specimen for fragments of X- or Y-chromosome is a common practice in legal medicine to verify the sex of an unknown perpetrator or victim, respectively. The correct classification of individuals into “female” or “male” is crucial for the success of criminal investigations. To avoid false classifications, the investigator must be aware of methodical pitfalls in sex determination, the problem of chimerism within one individual, and of possible discrepancies between the biological

gender and the (forensic relevant) “legal” gender^c appearing for example in the personal identification documents or in the registers of missing persons.

2. The methodical problem based on the amelogenin detection

In forensic casework the human amelogenin gene, which is located on both the X and Y chromosome as single copies in the XY homologous regions,¹ is widely used for sex determination by PCR in forensic routine analysis^{2,3} or anthropology.⁴ Development of highly discriminative multiplex-PCR systems with additional amelogenin detections makes the discrimination male/female rather easy and

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^c The term “legal” gender is used here merely as synonym for the gender appearing in official documents and registers as in the personal identity documents, the birth registers or the registers of missing persons; it is not intended to get into the ongoing discussion about the definition of “gender”.

was therefore very quickly included in forensic casework.^{5,6} No additional effort, lab time or costs were necessary. Sex determination was a kind of by-product of genetic fingerprinting. This easy-to-use method directly leads to the first possible pitfall in forensic sex determination:

In 1998, Santos et al. found a deletion of the amelogenin gene on the Y chromosome, which led to the disappearance of the usually longer Y-specific fragment and therefore to the detection of only the smaller X-specific signal, generating an apparently female genotype. According to their findings, they recommended, that a Y-specific locus should routinely be included in forensic sex tests to avoid false statements on gender determination.⁷ Maybe because their sample was rather small ($n = 24$), they did not receive large attention in the forensic community. However, this problem sporadically showed up in forensic casework.^{8,9} In 2002, two publications came out, also showing mis-typing of the Y-specific amelogenin gene by investigating a larger population number. The deletion of the AMEL Y gene has been reported in six out of 29,432 male individuals that were included in the Austrian Database, representing a deletion frequency of 0.02%.¹⁰ Thangaraj et al.¹¹ investigated 270 male of Indian origin and found false only female signals in five samples (1.85%) using the commercially available and well-used AmpF/STR Profiler Plus kit (Applied Biosystems). Regarding those findings, it was considered that gender determination in forensic casework can in some cases lead to wrong conclusions and should not rely on amelogenin typing alone.¹² Meanwhile, more investigations came up, strongly supporting the mentioned consideration: Recently, the IDF (Israel Defense Force) has reported on an amelogenin sex test failure on a phenotypically normal male soldier and also pointed to possible far reaching consequences in forensic trace analysis.¹³

3. The problem of chimerism

Difficulties in male/female discrimination may also arise after bone marrow transplantation as well as in the analysis of samples from women which are pregnant with a male fetus. In a bone marrow transplant, the patient's bone marrow is destroyed and replaced by healthy marrow/stem cells. In a successful transplant, the new bone marrow migrates to the cavities of the large bones, engrafts and begins producing normal blood cells. Usually, the patient receives bone marrow from a healthy donor with his or her own genetic pattern. Hence, after successful transplantation the forensically used short tandem repeats (STRs) found in the patient's blood are identical with the donor, but completely different from his/her own hair roots. Those were on the other hand identical to the patients original STR pattern. In DNA from buccal swabs or fingernails from the patient alleles of the donor and the patient were detectable five years after transplantation.¹⁴ In case of different sex between donor and recipient PCR based sex testing could therefore lead to wrong conclusions in forensic casework with regard to the gender. Meanwhile, there is

the possibility of using umbilical cord blood (UCB) as an alternative source of hematopoietic progenitors for allogeneic stem cell transplantation,¹⁵ which would not have any consequences from a forensic point of view. But today this method is still in the beginning.

Problems in sex determination could also arise when investigating biological blood traces from women which are pregnant with male fetuses. There is a time period during pregnancy in that the genetic material of the fetus (i.e., the Y-specific alleles) can be detected in blood of the mother.¹⁶ Assuming the mother as being the originator of blood in case of a missing person, it could easily be imagined that genetic analysis could possibly refer to blood from a male person and thus not from the missing woman.

4. Possible discrepancies between the biological gender and the (forensic relevant) legal gender

In forensic casework the diagnosis “female” or “male” has to be matched with data of missing or suspected persons by the police. These “reference” data comprise the officially registered (legal) gender, which is not necessarily identical with the genetic sex. One should keep in mind, that an X- or a Y-chromosome – be it detected with amelogenin or y-specific STR typing – does not necessarily tell the truth about the registered gender and the gender identity, i.e., the continuous and persistent sense of oneself as male or female. Usually, the gender is given to an individual at birth based on the phenotype unless a previous prenatal genetic investigation was done. This so-called “midwife-gender” is then filled in the birth certificate to become the legal gender. The phenotype based classification of the legal gender may contradict the genetical sex under several conditions as genetic diversity, intersex conditions, and transsexualism.

4.1. Genetic diversity/intersex conditions, particularly androgen insensitivity syndrome (AIS), gonadal dysgenesis (GD), and 5-alpha(2)-reductase-deficiency (ARD)

The sex chromosomes are involved in chromosomal abnormality relatively more common than are the autosomes. It can be calculated that at least 8% of all human conceptions have some form of chromosome abnormality. Most of them abort spontaneously while 0.5% can be found in liveborns. Half of the abnormalities involve the sex chromosomes, mostly trisomic states such as XXY, XYY and XXX, more rarely XO incidences.²⁸ Examples of genetic diversity, that lead to the described difference of genotype and phenotype and are therefore of forensic interest, are: Androgen Insensitivity Syndrome (AIS), Gonadal Dysgenesis (GD), and 5-alpha(2)-reductase-deficiency (ARD).

Complete androgen insensitivity syndrome (cAIS) is caused by mutations in the androgen receptor, producing impaired to complete lack of response to endogenous and

exogenous androgens. Individuals with complete AIS possess a normal male 46, XY genotype and functioning testes.¹⁷ According to their phenotype (female genitalia with a blind ending pseudo vagina and a lack of Müllerian duct structures) they are raised as girls and undergo normal breast development at puberty (if testes are in situ), but have complete absence of androgen dependent body hair in most cases.^{18,19} Individuals with partial AIS (pAIS) show ambiguous external genitalia with different degrees of masculinization, depending on the remaining capacity of receptors to react to androgens. Degree of masculinization and available operative techniques influences their upbringing as girls or boys, respectively.

GD is caused by varying mutations within the genetic cascade, which normally leads to testes differentiation (SRY, SF-1 or DAX1²⁰). It is characterized by abnormally formed gonads which were originally on the path to testis differentiation (gonadal streaks), female external genitalia, Müllerian duct development, and Wolffian duct regression. Female external genitalia develop due to the failure of the gonadal streaks to produce androgens necessary to masculinize. In pure GD (pGD) XY children are reared as girls, while in mixed GD sex assignment depends on genital masculinization, hence on remaining capacity of testes to produce androgens.

In individuals with ARD there is a lack of the enzyme which converts testosterone into dihydrotestosterone, which in turn is responsible for the formation of external male genitalia. Hence, these individuals, albeit they own XY-chromosomes, testes, müllerian structures and no wolffian ducts, are mostly raised as girls. The majority changes to the male gender during or post puberty, but some remain in the female gender, particularly if they are recognized in childhood, were subject to gonadectomy and receive oestrogen when puberty is to occur.²¹ Incidence of intersex conditions is still under discussion and depends on definition, methods and samples that are used: Estimation of incidence for AIS (complete and partial form) range from 1: 40,800²² to 1: 99,000,²³ while those for GD (pure and mixed form) and ARD remain unknown. Genel (2000) reported on eight subjects among 3.387 female athletes investigated during the Olympic Games in Atlanta showing a SRY-positive result in gender verification procedure, i.e., 1:423. In four cases this was due to a pAIS, in three cases due to cAIS, the remaining was caused by ARD.²⁵

4.2. Transsexualism

Individuals with gender identity disorder (GID; DSM-IV: 302.85) or transsexualism (TS; ICD-10: F64.0) have a strong and persistent cross-gender identification and a persistent discomfort with their sex or sense of inappropriateness in the gender role of that sex. Therefore, they live in the social role of the other sex and most of them undergo sex reassignment surgery. There are no recent epidemiological studies to provide data on prevalence of Gender Identity

Disorder. DSM-IV refers to data “smaller countries in Europe” with access to total population statistics and referrals suggest that roughly 1 per 30,000 adult males and 1 per 100,000 adult females seek sex-reassignment surgery” (APA, 1994, p. 535).²⁴

5. Conclusion and forensic relevance

In the field of forensic identification, DNA testing using STRs, and the detection of the amelogenin-specific fragments for sex determination is a commonly used method, usually without any or many regards to possible pitfalls. The use of Y-specific STRs has shown to be extremely useful in rape cases or deficient paternity investigations and are therefore used in many forensic labs.^{26,27}

However, since a false or misleading information about the classification of an individual as, female“ or, male“ will lead to serious consequences in criminal investigations (e.g., non-identification), the investigator must be aware of all possible pitfalls. The above named methodical pitfalls and problems in cases of chimerism can be overcome by adequate methodological approaches. In contrast, misinterpretations caused by discrepancies between the registered gender and the determined genetic sex cannot be avoided offhand. The practical relevance of this source of misinterpretation should not be underestimated.

In forensic casework all persons involved should be aware of the fact that, even though every possible methodical difficulties might be solvable, the determined sex is not necessarily legal gender.

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